

ON THE MORPHOLOGY OF THE CAUDAL GILLS OF
THE LARVÆ OF ZYGOPTERID DRAGONFLIES.

PART iii. (ONTOGENY), AND PART iv. (PHYLOGENY).

BY R. J. TILLYARD, M.A., B.SC., F.L.S., F.E.S., LINNEAN
MACLEAY FELLOW OF THE SOCIETY IN ZOOLOGY

(With fifteen Text-figures).

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INTRODUCTION.

In dealing with the Ontogeny of the Caudal Gills, it was found necessary to study, by means of sections, all instars of the growing larva up to the attainment of the complete internal form of structure. As this was found not to be reached until the seventh

instar, there was necessarily a considerable delay in raising the larvæ required from the egg, and in taking them successfully through all the stages to the seventh. It was for this reason that Parts iii. and iv. were not published with Parts i. and ii. in a single complete paper.

Even with the successful raising of several batches of larvæ to the seventh instar, it has turned out that not sufficient evidence has been obtained to throw much light upon one very interesting problem, viz., the origin of the constricted or two-jointed gill. The reason for this appears to be, that the only larvæ that I was able to raise were not of the Constricted Type. I had hoped that the gills of *Austroagrion cyane* Selys, which are classed as a Nodate Type, would offer some evidence, in their ontogeny, as to how the node developed. But, up to the seventh instar, this larva shows no sign of any division of the gill at all; so that one must evidently examine several more instars, before the beginning of the node would be made apparent. This meaning further delay, and the main objects of the paper having been in every other respect attained, I have judged it best to conclude my observations at this stage; being content to indicate, with regard to this one unsolved point, exactly how the problem stands, so that any student of Odonata, who is fortunate enough to obtain material, can pursue it to the end.

The results of Part iii. were obtained from the study of three species, viz., *Austroagrion cyane* Selys, *Ischnura heterosticta* Burm., and *Neosticta canescens* Tillyard. The larvæ of the first two were bred from the egg, and every instar up to and including the seventh (counting the pronymph as the first) was carefully studied by means of sections.* The *Neosticta* larvæ, belonging probably to the fourth and fifth instars, were only a fortunate find; not having been raised from the egg, their instars cannot be stated with absolute certainty.

I need add little to the account of the methods employed, as

* As the series of sections obtained from *Austroagrion* were better than those obtained from *Ischnura*, I have figured only the former. *Austroagrion* has nodate gills, *Ischnura* subnodate; but, up to the seventh instar, the two forms show no important differences.

given in Part i. of this paper (These Proceedings, 1917, Vol. xlii., Part i., pp.39-44). The method of double-embedding is *essential* for these tiny structures, and every detail must be carried out with the greatest care, if one would have a final result of any value at all. The best results were those that, after staining, were de-stained back almost to the lightest possible limit. Such preparations should, of course, be fully studied at once, unless one is willing to risk the chance of their fading before the work is completed.

In Part iv., the results obtained in Part iii. are considered in conjunction with those obtained from a study of the caudal filaments of the Perlaria and Plectoptera, the combined evidence from all those sources giving us some valuable phylogenetic results.

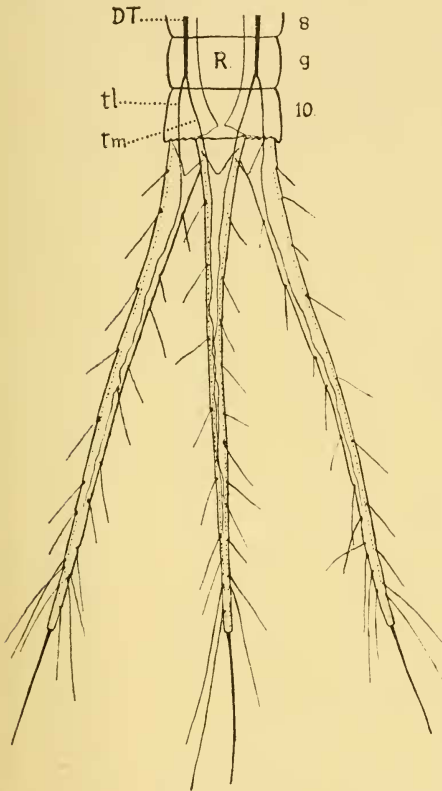
Part iii. ONTOGENY OF THE CAUDAL GILLS.

Structure of the Gills in the newly-hatched larva or Second Instar.
(Text-figs. 33, 34).

Text-fig. 33 shows the external form of the gills in the newly-hatched larva of *Austroagrion cyane* Selys, which is typical of all Vertical Lamellar Gill-types. The gills are elongated, slender filaments, clothed with short, stiff hairs externally, for most of their length, but carrying, near the tip, a few longer and more pliable hairs. The tip itself is bluntly pointed, and carries a long, stiff, bristle-like hair. The gills are quite transparent and without pigmentation. The median (dorsal) gill possesses two main longitudinal tracheæ, one derived from each dorsal trunk of the abdomen. The lateral gills, however, each possess only one main longitudinal trachea, derived from the dorsal trunk of its own side. The tracheæ do not reach as far as the tip of the gill.

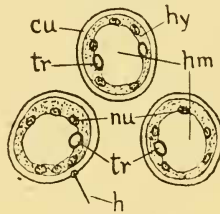
In transverse section, the gills are nearly circular (Text-fig. 34). The cuticle is thin, and is followed, on its inner side, by the almost equally thin hypodermis, which shows only from four to six nuclei in any given section. The interior of the gill consists of an undifferentiated blood-space, or portion of the hæmocœle. In the median gill, the two main tracheæ lie mid-laterally, one on either side, close to the hypodermis. In each lateral gill, the

single main trachea lies latero-ventrally, on the inner side of the gill. Neither definite blood-canals, internal laminae, nor alveolar meshwork are developed.



Text-fig. 33.*

The nerve-supply can be followed with difficulty from the eighth ganglion to the bases of the gills, but cannot be seen in sections of the gills themselves, probably owing to its extreme fineness. It is clear, however, that the median gill receives two main nerves dorso-laterally at its base, while



Text-fig. 34.†

each lateral gill receives only a single nerve placed somewhat ventrally. As it is impossible to follow the changes

in the nervous system in such tiny organs as these gills, I shall not mention them further, until they come plainly into view in much larger gills.

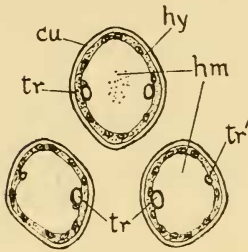
* Caudal gills of *Austroagrion cyane* Selys, second instar; dorsal view; ($\times 90$).

† T.S. through caudal gills of *Austroagrion cyane* Selys, second instar, at about one-third from base; *cu*, cuticle; *h*, hair; *hm*, haemocoel; *hy*, hypodermis; *nu*, nucleus of same; *tr*, trachea; ($\times 370$).

Structure of the Gills in the Third Instar.

(Text-fig.35).

The gills are still filiform, and resemble those of the second instar very closely; the principal difference being that, in each lateral gill, a branch-



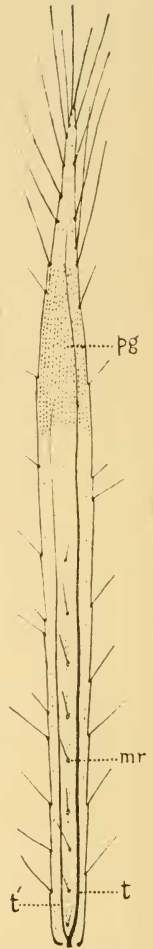
Text-fig.35.*

trachea of small calibre is developed from the base of the main trunk, and runs for some distance up the gill. This branch lies on the outer side of the gill, a little more dorsally placed than the larger trachea from which it is derived. It is very difficult to make it out in sections, as it is usually completely collapsed. The main trunk now lies very little below mid-laterally on the inner side of the gill, and projects very definitely into the hæmocoel. It also reaches nearly to the gill-tip.

Structure of the Gills in the Fourth Instar.

(Text-figs.36, 37).

In this instar, we notice the first external sign of a change from the filiform to the lamellar type of gill, slight though it be. For at least half of its length, the gill remains filiform. But the distal half shows a distinct, though very slight, attempt at flattening and widening out, so that the gill becomes shaped as shown in Text-fig.36. In the lateral gills, the branch-trachea is now very distinct, being at least half the calibre of its parent-trunk, and running along more than three-fourths of the length



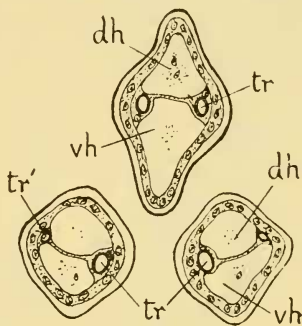
Text-fig.36.†

* T.S. through caudal gills of *Austroagrion cyane* Selys, third instar, at about one-third from base. Lettering as in Text-fig.34; *tr'*, branch-trachea from near base of main trachea (*tr*) in lateral gills; ($\times 370$).

† Lateral caudal gill of *Austroagrion cyane* Selys, fourth instar; side-view; ($\times 90$).

of the gill. In *Austroagrion cyane*, but not in *Ischnura heterosticta*, this stage is marked by the presence of a distinct pigment-zone on the distal fourth of the gill.

Sections of the gills at this stage show some important developments internally (Text-fig.37). Their form, in the basal half, now begins to approach the Triquetro-quadrate Type; while, in the slightly widened and flattened distal half, they have taken on, in cross-section, a distinctly oval form, which is the preliminary step to the differentiation of the rhachis and blade of the true Lamellar Type. The tracheæ are now much larger, even the second longitudinal trachea of the lateral gills being easily made out. They project definitely into the hæmocœle, and both lie at the mid-lateral level. But, most important of all, is the division of the hæmocœle into two parts, by an outgrowth of the hypodermis forming a definite internal lamina across its cavity, between the two main tracheæ. This is the first differentiation of the dorsal and ventral blood-canals.



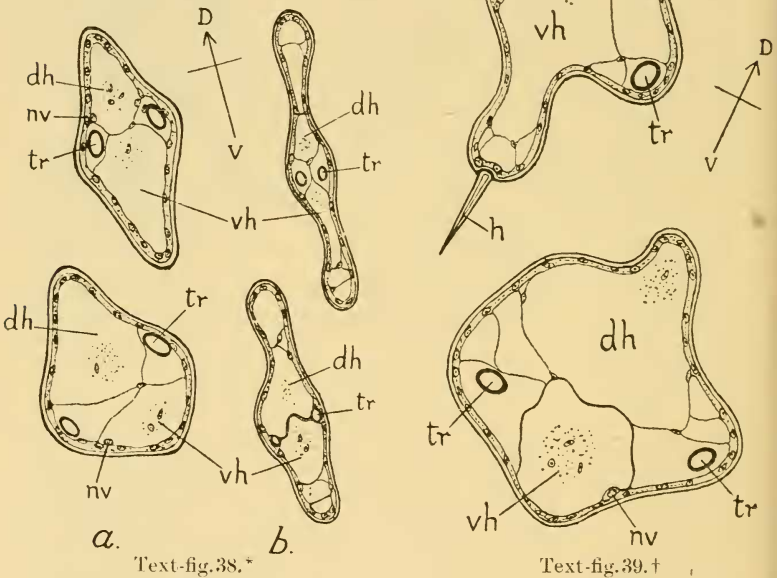
Text-fig.37.*

Structure of the Gills in the Fifth Instar. (Text-fig.38).

Externally, there is only a slight increase in the width of the distal half of the gill, so that the difference in shape from that shown in Text-fig 36 is scarcely appreciable. Internally, the differentiation of the hæmocœle has proceeded one step further. The internal lamina has split up to form the inner boundaries of four separate divisions, viz., the dorsal and ventral blood-canals, and two smaller divisions to enclose the two main tracheæ respectively. It is important to notice here that, in the median gill, the dorsal blood-canal is smaller than the ventral, while, in

* T.S. through the caudal gills of *Austroagrion cyane* Selys, fourth instar, at about one-third from base; *dh*, dorsal blood-canal; *tr*, main trachea; *tr'*, branch-trachea destined to become second main trachea in lateral gills; *vh*, ventral blood-canal; ($\times 370$). [N.B.—In this instar, the division of the hæmocœle does not extend much beyond half-way].

the lateral gills, the opposite is the case. Thus the blood will flow faster dorsally than ventrally in the median gill, but faster ventrally than dorsally in the lateral gills. Therefore, in each case, the afferent blood-stream is the faster of the two, flowing along the narrower channel. Thus arises the first sign of a differentiation between the primary or afferent blood-canal, and the secondary or efferent blood-canal.



In Text-fig. 39, I have shown a transverse section across the gills of a small larva of *Neosticta canescens* (Saccoid Type), which

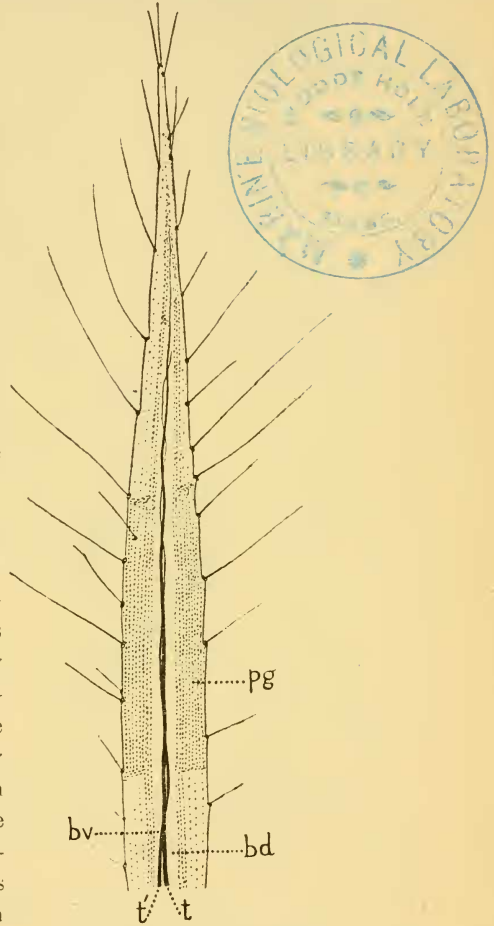
* Two T.S. through median and left lateral caudal gills of *Austroagrion cyane* Selys, fifth instar, *a* at about one-third from base, *b* at beyond half-way along the gill. Lettering as in Text-fig. 37; *nv*, main nerve; the arrow VD shows the direction of the sagittal plane, the median dorsal gill being uppermost; ($\times 370$).

† T.S. through median and right lateral caudal gills of *Neosticta canescens* Tillyard, fourth or fifth instar. Lettering as in Text-figs. 37 and 38; *h*, hair; ($\times 370$).

I judge to be either of the fourth or fifth instar. The differentiation of the hæmocoel is much more clearly shown in this type, the primary or afferent blood-canal being much smaller, more regularly formed, and more strongly walled than the secondary; the latter indeed appears to be merely the large remainder of the hæmocoel left over after the tracheæ and the primary blood-canal have been closed off. The beginnings of the alveolar meshwork can be clearly seen within it. It is much to be regretted that I could not obtain a series of this very rare, archaic, larval type, so as to follow out the changes in the blood-canals more fully.

Structure of the Gills in the Sixth Instar. (Text-figs.40, 41).

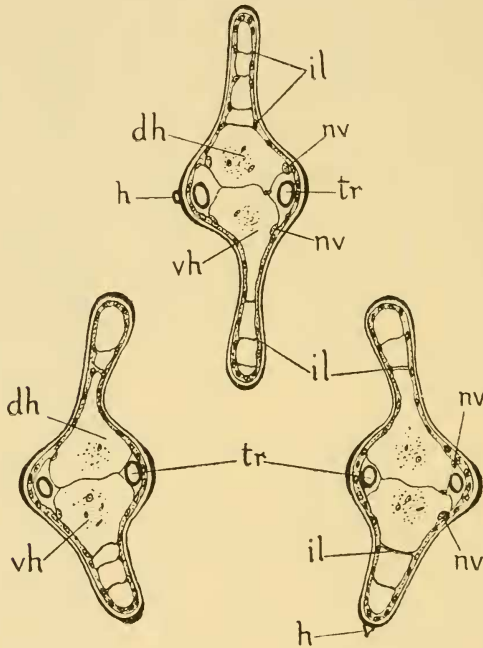
At this stage, the gill is definitely flattened for about two-thirds of its length, though still very narrow (Text-fig.40). The blood-canals are distinguishable in the whole gill, particularly where they run through the pigment-zone in *Austroagrion cyane*. Transverse sections now begin to show the definite differentiation into rhachis and blade, as may be seen from Text-fig.41, which is taken from a little beyond half-way along the gill. At this stage, the



Text-fig.40.*

* Distal expanded portion of a lateral gill of *Austroagrion cyane* Selys, sixth instar, side-view; *bd*, dorsal blood-canal; *bv*, ventral blood-canal; *pg*, pigmented zone; *t*, main trachea; *t'*, branch-trachea, which has become the second main trachea; ($\times 90$).

primary and secondary internal laminae begin to develop. It is also possible, in some sections, to make out the main longitudinal nerves, which do not appear to differ in position from those of the gills in the fully grown larva.



Text-fig. 41.

T.S. through caudal gills of *Austrocyprion cyane* Selys, sixth instar, at about half-way along the gill. Lettering as in Text-fig. 37; *h*, hair; *il*, internal lamina; *nv*, main nerve; ($\times 370$).

Structure of the Gills in the Seventh Instar.

In this stage, the gills only differ from those of the Sixth Instar by their slightly broader lamellar form, and by the more definite formation of the internal laminae, together with the first beginnings of the alveolar meshwork. Having reached this stage, the gill contains all its essential structures. Further de-

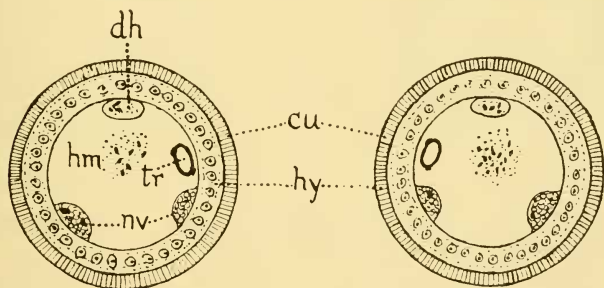
velopment consists only in the broadening of the blade of the lamella. With this is correlated the formation of the rich tracheal branchings into the blade, which are such a conspicuous feature of the gills in older larvæ.

Part iv. PHYLOGENY OF THE CAUDAL GILLS.

In attempting to work out the Phylogeny of the Gills, it is legitimate not only to seek for the evidence afforded by their Ontogeny, but also to turn to the study of the homologous organs in those Orders most closely related to the Odonata. Since the Caudal Gills of Zygoptera are the modified cerci and appendix dorsalis of the larva, and since the only other Orders that can come into the question are the Perlaria and Plectoptera, we must turn to the study of the *caudal filaments* of their larvæ.

The Caudal Filaments of the Perlaria. (Text-fig.42).

The larvæ of the Stone-Flies or Perlaria possess only two caudal filaments, viz., the *cerci*. These are, therefore, the homologues of the two lateral gills of the Zygoptera. They are usually



Text-fig.42.

T.S. through the two caudal filaments of a well-grown Perlid larva, sub-family *Leptoperlinæ*; *cu*, cuticle; *dh*, dorsal blood-canal; *hm*, hæmocœle; *hy*, hypodermis; *nv*, main nerve; *tr*, main trachea; ($\times 120$).

very long and many-jointed, but there are not lacking a number of genera in which a great reduction in length, and also in the

number of joints, points the way by which the cerci of the Zygopterid larva arrived at its present one- or two-jointed state.

In transverse section, these organs are absolutely circular. The cuticle and hypodermis are thick, the nuclei of the latter numerous and closely-placed. The interior of the organ is a hollow blood-space, or extension of the hæmocœle, showing the presence of both plasma and corpuscles in almost every section. Laterally, on the outer side, close to the inner border of the hypodermis, there is a single main trachea. Latero-ventrally, on either side, seated upon the inner border of the hypodermis, there are two large longitudinal nerves, whose cross-section is greater than that of the trachea. As the caudal filaments of the *Perlaria* are organs of touch rather than gills, the greater size of the nerves, and the smallness of the trachea, in comparison with those of Zygopterid gills, is only to be expected.

The most interesting structure in these caudal filaments is the very small but definite dorsal blood-vessel, situated mid-dorsally below the inner border of the hypodermis. This structure has a well-defined wall, and might be mistaken for a trachea, were it not always quite full of blood-plasma and corpuscles. It continues far along the filament, opening distally into its main interior, *i.e.*, the hæmocœle.

The comparison between these organs and the lateral caudal gills of Zygoptera may be summarised as follows:—

(1) Only the afferent or primary blood-canal is marked off in the *Perlaria*; but it is much smaller and more definitely separated out than in the Zygoptera; moreover, it is placed mid-dorsally, as in the *median* gill of Zygoptera; whereas, in the lateral gills of the latter, the afferent canal is ventral.

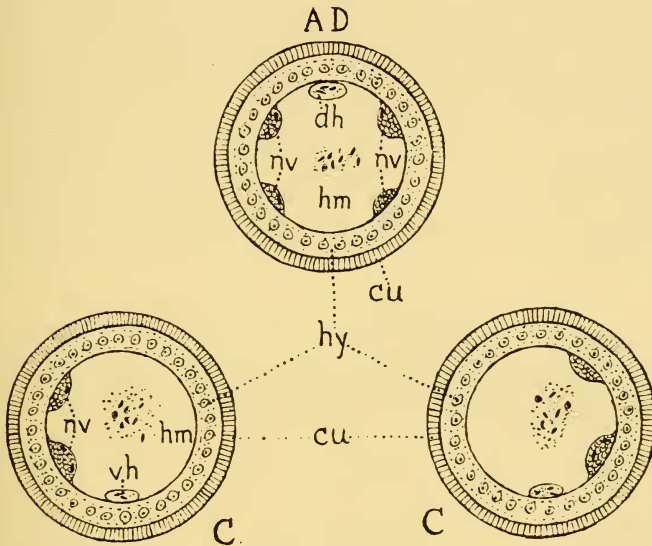
(2) Only one main trachea is developed, on the inner side of the hæmocœle. Thus the tracheation remains at the same stage as that shown in the Second Instar of Zygopterid larvæ (Text-fig. 34).

(3) Two main longitudinal nerves are developed, as in the lateral caudal gills of Zygoptera; but their position is not quite the same, both being ventrally placed, whereas those of the Zygoptera lie both on the outer side, one ventral and one dorsal.

The Caudal Filaments of the Plectoptera. (Text-fig.43).

The larvæ of the May-Flies or Plectoptera possess three caudal processes, viz., the two latero-ventral *cerci*, and the single median dorsal *appendix dorsalis*. Thus the former are the homologues of the lateral caudal gills of the Zygopterid larva, while the latter is the homologue of the median caudal gill.

All three of these filaments closely resemble those of the *Perlaria* externally, being long, slender, and many-jointed.



Text-fig.43.

T.S. through the three caudal filaments of a well-grown Plectopteran larva, *Atalophlebia* sp. Lettering as in Text-fig.42; AD, appendix dorsalis; C, cercus; *vh*, ventral blood-canal; ($\times 130$).

In transverse section (Text-fig.43), the structure of these organs is of great interest. In all three, the transverse section is circular, the cuticle and hypodermis fairly thick, the latter with many closely-arranged nuclei, and the interior an open prolongation of the hæmocœle, as in the filaments of the *Perlaria*. Beyond this, the cerci differ so much from the appendix dorsalis that we must deal with them separately.

Cerci.—Comparing these with the cerci of *Perlaria*, we see that both possess two main nerves and a single closed afferent blood-canal. But, in the *Plectoptera*, the closed blood-canal is ventral in position, *as in the lateral caudal gills of Odonata*, and not dorsal, as in the *Perlaria*. The two main nerves also lie in the same positions as in the *Zygoptera*, and not as in the *Perlaria*. There is no main longitudinal trachea developed at all in the cerci of the *Plectoptera*.*

Appendix Dorsalis.—As this is absent in the *Perlaria*, we must confine our comparison to the median caudal gill of the *Zygoptera*. We then notice a remarkable similarity in the two homologous organs. *For the closed afferent blood-canal is dorsally placed in both, and both possess four main nerves, two being latero-dorsal, and two latero-ventral*. The only differences are that there is no main trachea in the appendix dorsalis of the *Plectoptera*, and no development of internal laminae or alveolar meshwork.

Phylogenetic Conclusions.

The following conclusions may, I think, be safely drawn from the combined evidence of the Ontogeny and the comparison with the homologous organs of the *Perlaria* and *Plectoptera*.

(1) *The relationship between the Perlaria, Plectoptera, and Odonata.*

Firstly, as regards the *Perlaria*, there are some fundamental differences in the arrangement of the internal structures of the cerci, as compared with those of the *Zygoptera*. These are (*a*) the position of the main nerves, and (*b*) the dorsal position of the closed afferent blood-canal. I think that these differences are great enough to make us conclude that the evidence from the cerci only reinforces the opinion which I had previously stated,† from a consideration of other morphological characters, viz.,

* This applies to *Atalophlebia*, the only genus available here for study. I cannot say whether it is true for the whole Order or not.

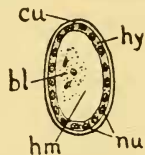
† "The Biology of Dragonflies." Cambridge University Press, 1917, pp.5-6.

that no close relationship exists between the Perlaria and the Odonata

Secondly, as regards the Plectoptera. The close resemblance in the arrangement of the internal structures of the caudal processes, as compared with those of the caudal gills of Zygoptera, is indeed a striking one, since the number and position of the main nerves, and the positions of the primary afferent blood-canals in the three processes are the same in both. The only differences are the long, many-jointed condition of the filaments in Plectoptera, and the absence of a main longitudinal trachea (assuming that this character holds for all Plectoptera). We are, I think, bound to conclude that the relationship between the Plectoptera and Odonata is a more definite one than that between the Perlaria and Odonata, and that, in particular, the Zygopterid gill has been derived from a caudal process closely similar to that of the Plectoptera, by reduction from a many-jointed to a one- or two-jointed condition.

(2) *Steps in the evolution of the Zygopterid Gill.*

If we examine in section any simple evagination of the body-wall of an Insect Larva, forming a short process—such as, for instance, the *cercoids* of the Odonate Larva—we shall find that they show only cuticle, hypodermis, and internal blood-space (Text-fig. 44). No longitudinal nerves, tracheæ, or closed blood-canals are present. Thus the first stage in the evolution may be assumed to have been a many-jointed process, long, slender, circular in section, with fairly stout cuticle and hypodermis enclosing an undifferentiated blood-canal, and without either main nerves, tracheæ, or closed afferent blood-canals.



Text-fig. 44. *

The next stage would be the development of this organ into an organ of touch, by the outgrowth of longitudinal nerves along

* T.S. through a cercoid of the well-grown larva of *Synlestes weyersi* Selys; *bl*, blood; *cu*, cuticle; *hm*, hemocoel; *hy*, hypodermis; *nu*, nucleus of same; ($\times 88$).

it, as branches of the tenth-segment nerves of the abdomen. Probably correlated with this would be the regulation of the blood-circulation, by the development of a closed afferent canal, the *primary blood-canal*. As the positions of both these structures are different in Perlaria and Odonata, it is logical to assume that the two Orders became separated at a stage in the evolution of the Insect Larva earlier than is here indicated.

The third stage would be the development of the main trachea along the interior of the processes, the cerci receiving one each, from the dorsal trunk of its own side, but the median appendix dorsalis receiving two, one from each side. Unless these tracheæ can be shown to exist in some Plectopterous larvæ, we must hold that the Odonata became differentiated from their common stock with the Plectoptera before this outgrowing of the tracheæ took place.

Finally, as all the appendages of the Odonata, in every part of the body, show a great reduction in the number of joints (*e.g.*, the antennæ, the tarsal joints, the labial and maxillary palpi), we must assume that the reduction of the caudal processes to a one- or two-jointed form proceeded parallel with these other reductions; in other words, the original Protodonate ancestor already possessed all these reductions, derived from a Palæodictyopterous ancestor.

Thus we conclude, that the original form of Zygopterid caudal process was a short, one- or two-jointed appendage, in which the nerves and blood-canals were developed as in the caudal processes of the Plectoptera, and with the addition of a single longitudinal trachea in each cercus, and two in the appendix dorsalis. In this organ, neither internal laminæ nor alveolar meshwork were developed.

Evolution of the Caudal Gills within the Suborder Zygoptera.

We have now to consider the evidence of the Ontogeny, with respect to the further evolution of gill-types within the Suborder.

As far as I can ascertain, all newly-hatched Zygopterid larvæ have the gills filiform, one-jointed, and hairy. We may safely assume that the filiform condition is primitive, since all the evi-

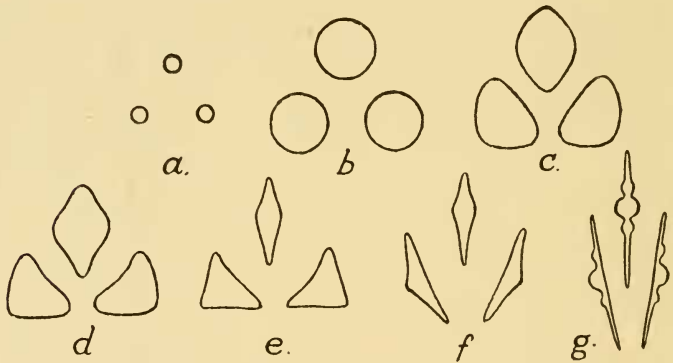
dence gathered in the preceding section points also in this direction. Probably also the hairiness is primitive too, since all aquatic groups tend to become smooth in the course of evolution. But the one-jointed condition may not be taken as primitive: because, in the case of other organs, *e.g.*, the antennæ, tarsi, etc., the number of joints increases as the larva grows, and yet it is certain that the last and highest number of joints attained is the most primitive number, or, in some cases, even less than that (*e.g.*, in the tarsi, the fossil *Tarsophlebiinæ* had four joints, whereas no existing Odonate has more than three). Thus we must assume that, in the case of a larva that develops two-jointed gills, such as *Neosticta*, the two-jointed condition is probably primitive. But, in the case of those larvæ which always have unjointed gills, it is not necessary to assume that they are derived from two-jointed forms, unless there is evidence for it.

In the case of the gills of *Calopterygidae* and *Lestidae*, there is absolutely no evidence that these gills were ever more than one-jointed. But in the *Agrionidae*, the existence of two-jointed forms in the *Protoneurinae* and *Agrioninae* strongly suggests that the common ancestor of these two groups had a larva with two-jointed gills. As no larvæ of the most primitive subfamily of the *Agrionidae* (*viz.*, the *Megapodagrioninae*) are known to possess two-jointed gills, I am inclined to suspect that the original *Agrionidae*, like the other two families of *Zygoptera*, possessed only one-jointed gills, and that the two-jointed condition arose in the common ancestor of only those subfamilies still possessing it, and, failing to become of any definite service to the larva, is now again reverting to the one-jointed form, by degradation through the Nodate, Subnodate, and finally Denodate stages.

The most ancient existing forms of *Zygoptera* possess gills of the Saccoid Type; for this type appears to be universal throughout the two subfamilies *Epallaginae* and *Thorinae*. As such a type could be easily produced from an original caudal filament, by simple enlargement of its internal cavity, we shall probably be right in assuming that the Saccoid Gill is the most primitive type. One might expect this line of evolution, once begun, to run to an extreme; and this is probably the reason for the exist-

ence of the huge, cumbersome, bladder-like gills of *Diphlebia* and *Cora*. It seems clear, also, that these organs are primarily an attempt to evolve *blood-gills*, and not *tracheal-gills*; since it is the blood-space that undergoes an immense increase in volume,—the tracheal supply remaining far inferior, in these gills, to that to which it eventually attains in the Lamellar Gill.

Although we designate the Saccoid Type as the most archaic of all, there is one qualification to add. These huge bladder-like gills could not be held apart in the water. The two lateral ones must always have rested either on the river-bed, a rock, or a convenient stem, on which the larva may have been resting.



Text-fig. 45.

Evolution of the outward form of the caudal gills, as shown in T.S. Phylogenetic series from caudal filament, through simple Saccoid and Triquetro-quadrante Types, to Lestid form of Vertical Lamellar Type; *a*, caudal filament; *b*, simple saccus; *c*, *Diphlebia*-stage of same; *d*, *e*, evolution of the Triquetro-quadrante Type (*e*); *f*, *g*, evolution of the Lestid form of Vertical Lamellar Type (*g*).

The median gill must also have rested symmetrically upon the other two. Hence, even in the huge bladder-like gills of *Diphlebia*, the *beginnings* of the Triquetro-quadrante Type are evident. That type, as seen in its highest development in the *Calopteryginae*, is clearly a specialised development from the older Saccoid Type (Text-fig. 45, *a-e*). Thus we may say that a single line of

evolution, from the archaic Saccoid Type to the highly specialised Triquetro-quadrate Type, characterises the family *Calopterygidae* as a whole.

From the Triquetro-quadrate Type of the *Calopterygina*, the Lestid form of Lamellar Gill-Type is undoubtedly derived. This can be seen in Text-fig. 45, *e-g*, where I have shown diagrammatically the probable stages in the reduction-process. In connection with this, the following points are of importance:—

(1) There is no evidence of the *Lestidae* ever having possessed the division of the blood-canals into two, in their lateral gills, as seen in these gills in the *Calopteryginae*. This must be regarded as a specialisation confined to that subfamily.

(2) There is strong evidence that the Lestid gill-form is derived from a form in which the tracheation was the same as in the Triquetro-quadrate Type:—

For (*a*), regenerated gills of Lestid larvæ always show an increase in the number of tracheæ; and, in most cases, this increase takes the form of a doubling of the original number of tracheæ, and therefore agrees with the original Triquetro-quadrate arrangement; and (*b*) the reduction of the number of main nerves in the median gill in the *Lestidae* is unique in *Zygoptera*, and only to be explained as a specialisation from the original arrangement of four nerves in the median gill and two in each of the laterals.

Within the *Lestidae*, it is clear that the gills of the *Synlestinae* are the more primitive type; for (*a*), regenerated gills of the subfamily *Lestinae* assume the *Synlestinae* form, with the branch-tracheæ obliquely placed in the main stem; and (*b*), the arrangement of these branch-tracheæ at right angles to the main stem in *Lestinae*, and the development of the pigment-bands, are evidently high specialisations, the former being unique in the Sub-order.

The evidence of the structure of the Caudal Gills, then, tends to bring the *Lestidae* into closer relationship with the *Calopterygidae* than a study of the imaginal characters alone would warrant. In particular, it would seem that the larvæ of the *Calopteryginae* and *Synlestinae* are very closely allied.

Turning next to the *Agrionidae*, we have a very distinct problem to deal with. I think that the evidence here is all in favour of the development of the Agrionid form of Vertical Lamellate Gill-type direct from a two-jointed or constricted Saccoid Type, such as still exists in *Neosticta*, without the intervention of a *Triquetro-quadrate Type* at all. The reasons for this are as follows:—

(a) *Neosticta* can hold its gills fairly widely apart, and they are not swollen out so greatly (as in the case of *Diphlebia*) that the lateral gills would be compelled, in time, to take on a triquetral form, by pressure of the median gill from above and between them. Consequently, there is an opportunity for the development of a quadrate form in all three gills.

(b) The gill being two-jointed, such a quadrate form naturally suggests itself as suitable for the basal joint or *stalk* of the gill.

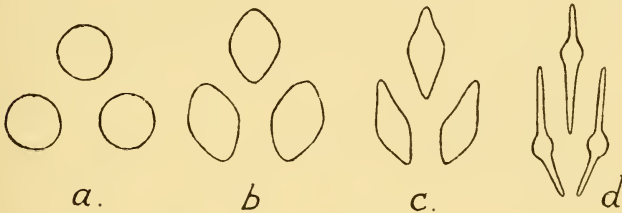
(c) All three gills of the Agrionid form of Vertical Lamellar Gill-Type *do actually show this quadrate form* in transverse section. (See Text-fig. 22 in Part ii. of this paper, p. 90).

(d) The internal arrangement of parts in these gills is that of the Saccoid Type, except only that the median gill possesses *two* main tracheæ, instead of four. That this is due to reduction is shown by the fact that regenerated median gills of the Agrionid form of Vertical Lamellar Gill-type show an increase in the number of main tracheæ to *four*, as well as a distinct tendency to a narrow Saccoid form. (N.B.—Regenerated lateral gills also show an increase to three or four tracheæ, but *three* main tracheæ are also quite frequently met with in *Neosticta*, as well as in other Saccoid Types).

The Constricted or two-jointed Lamella of *Isosticta* is clearly a direct development from the Constricted Saccus of *Neosticta*. The two genera are too closely allied to admit of any doubt on that point.

The larva of *Caliagrion billinghami*, when half-grown, (probably the tenth or eleventh instar) shows the constricted condition almost as completely as in *Isosticta*. Thus the Nodate form finally attained in this and other Agrionine genera must be derived from the older Constricted form, as seen in *Isosticta*.

But we may not argue from this that the *Agrioninae* are descended from the *Protoneurinae*, since such a supposition is negatived on all other morphological grounds. The wing-venation of the *Protoneurinae* is reduced far beyond the *Agrionine* stage. It is quite possible that the larvæ of some archaic *Agrionine* genus may still retain gills of constricted form. We can only say that such a form must have existed once within the *Agrioninae*, even if no longer existing to-day. The phylogenetic series of gill-types (Constricted Saccus, Constricted Lamella, Nodate Lamella, Subnodate Lamella, Denodate Lamella) is strictly correct. But it does not follow that the series of generic types which to-day exhibit these gill-types (e.g., *Neosticta*, *Isosticta*, *Caliagrion*, *Ischnura A gioenemis*) is a true phylogenetic sequence. The sequence *Neosticta*, *Isosticta* must be admitted. Then there is a break; for, as far as we know, the larvæ of the



Text-fig. 46.

Evolution of the Agrionid form of the Vertical Lamellar Type direct from the Constricted Saccoid Type; a, Saccoid Type; b, c, intermediate stages; d, Agrionid form of Vertical Lamellar Type.

Protoneurinae have reached no higher stage than that shown by *Isosticta*. There is also a break on the side of the *Agrioninae*; for, as far as we know, no larva of this subfamily possessing vertical lamellar gills shows any older form than the Nodate of *Caliagrion*. Thus the constricted stages still present in the larvæ of *Protoneurinae* have been *passed and lost* by the more highly specialised larvæ of the *Agrioninae*; so that it is pure chance that *Caliagrion* picks up the sequence of forms at the stage next beyond that to which *Isosticta* has attained. There

might just as well have been a gap, or an overlapping, in the two subfamilies.

Within the subfamily *Agrioninae*, we can follow the phylogenetic sequence of gill-forms from the Nodate, through the Subnodate, to the Denodate, which reaches its final form in the tough, thickened, opaque gills of *Austrocnemis* and some species of *Agrion*. From such a type as this, a retrogression due to loss of function, (the gills no longer serving as breathing-organs) will explain the presence of the Reduced (Non-functional) Type in such a highly specialised case as the larva of *Agrion asteliv*, which dwells in the water collected at the bases of the leaves of certain plants, in a region of high rainfall.

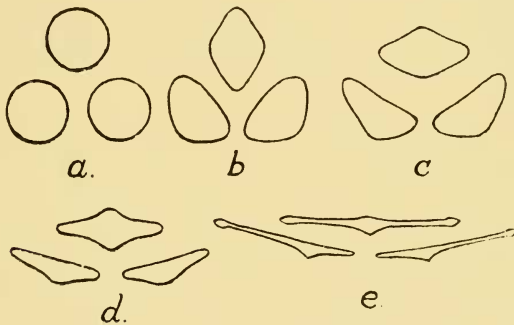
Text-fig.46 shows diagrammatically the stages in the development of the Agrionid form of Vertical Lamellar Gill from its Saccoid ancestor.

There remains now only the Horizontal Lamellar Type of gill to deal with, as we see it developed in the genus *Argiolestes*. It seems to me that the development of the two types of Lamellar Gills, vertical and horizontal, is obviously a case of the accumulated result of habit on the form of an organ.* If the original Saccoid Gills were held out separately in the water, as in the case of *Neosticta*, then, in course of time, it is inevitable that the Vertical Lamellar Type must be developed. But if, on the other hand, the ancestral form, possessing a Saccoid Gill-system, remains a rock-dweller, and adopts the habit of clinging closely to the rock-surface to escape detection, then the natural result must be, in course of time, that the gills will become horizontally flattened. They will, therefore, pass through a triquetro-quadrate stage, represented in Text-fig.47, *b, c*, and this stage must be observable in a difference between the form of the lateral and median gills. The gills of *Argiolestes* do show this difference, though slightly; for the lateral gills are completely triquetral in section, while the median gill still preserves the quadrate shape,

* Since the organs with whose evolution we are dealing are purely larval structures, not present in the imago, this appears to be a very interesting problem for the consideration of Lamarckians and Darwinians.

especially near the base of the gill. Text-fig.47 shows diagrammatically the derivation of the Horizontal Lamellar Type from the Saccoid Type.

The gills of *Argiolestes* are composed of a single joint. There is nothing in their structure to suggest that they are derived from two-jointed, ancestral forms.* I think, therefore, that the archaic subfamily *Megapodagrioninae*, to which this genus belongs, will prove (when its larvæ are known) to possess, throughout, one-jointed gills, and that most of these are more likely to be saccoid, triquetro-quadrate, or vertical lamellar types, rather than of the highly specialised form found in our Australian *Argiolestes*.



Text-fig.47.

Evolution of the Horizontal Lamellar Type; *a*, saccoid type; *b*, *c*, *d*, successive stages in the horizontal flattening; *e*, horizontal lamellar type.

The number of forms studied in this paper, particularly in the case of the extensive family *Agrionidae* (within which, not a single representative of either of the two subfamilies *Pseudostigmatinae* and *Platycnemidinae* is available in Australia for study), is far too small for us to draw any very definite conclusions as to the course of evolution of the various groups within the Sub-

* Except, perhaps, the peculiar form of the regenerated gill in *Argiolestes griseus*; see Part ii. of this paper, Plate i., fig.11.

order. But there are certain conclusions in which the evidence from the structure of the Caudal Gills reinforces that already gained from the study of other larval and imaginal organs; and these, I think, may by now be considered as reasonably proved. They are as follows:—

(1) Of the three families, the *Calopterygidae* are the oldest. Within the *Calopterygidae*, the *Epallaginae* on the one hand, and the *Thorinae* on the other, remain primitive; but the *Calopteryginae* must be reckoned as considerably more specialised.

(2) The *Lestidae* appear as a very distinct family, apparently derived from ancestors having close affinity with *Calopterygidae*. If we could obtain the larva of *Epiophlebia*, it is probable that some valuable evidence would be forthcoming as to the more exact origin of this interesting family. The evidence of the Caudal Gills, in any case, reinforces that of the venation on the point that the *Lestidae* are most certainly distinct from the *Agrionidae*.

(3) The *Agrionidae* are the dominant family, and contain the most highly specialised types of the Suborder. But the *Megapodagrioninae* remain still archaic, and far removed from the higher forms. The larval development of *Argiolestes* is specialised along a line quite unique within the Odonata. The two-jointed gills offer strong evidence in favour of the origin of the *Protoneurinae* and *Agrioninae* from a common ancestor, whose larval form must have closely resembled that still extant in the former subfamily. As the *Platycneminae* (about whose larvæ little is known) clearly connect the *Protoneurinae* on the one hand, and the *Agrioninae* on the other, with the archaic *Megapodagrioninae*, it is to be hoped that the study of their larvæ will soon be undertaken.

Apart from the Phylogeny of the Gill-System as a whole, it would be advisable to state briefly the stages through which the different internal structures of the gills have passed:—

(1) *The Blood-System*:—Originally a simple extension of the hæmocœle into the caudal process, this early became differentiated by the closing-off of a narrow canal for the afferent blood-flow. We have already given reasons for supposing that

this *primary blood-canal* existed in the earliest Odonata. When the caudal process swelled out into the Saccoid Type of Gill, a great increase must have taken place in the size of the hæmocoel within it; and this increase must have affected the afferent canal, as well as the rest of the blood-space. At this stage, the efferent or return-current must have proceeded sluggishly back along the undifferentiated portion of the hæmocoel. The subsequent development of the *internal laminae* and the *alveolar meshwork*, within this undifferentiated portion, closed-off the *secondary* or *efferent canal*, which we have seen, from the ontogenetic evidence afforded by an early instar of *Neosticta*, is larger and less definitely developed than the older primary or afferent canal. The final result is that the original hæmocoel of the gill becomes divided up into two distinct canals, surrounded by a mass of alveolar tissue, with zones of internal laminae.

(2) *The Nervous System*:—The original Odonata must have possessed caudal processes with a nerve-supply similar to that found in the Plectoptera, viz., two main nerves in each cercus (both on the outer side), and four in the appendix dorsalis (two dorsal and two ventral). This remains the same throughout all subsequent developments of gill-types, except only in the case of the Lestid form of the Vertical Lamellar Gill-Type, in which the median gill has its nerve-supply reduced to two only, by the suppression of the two ventral nerves.

(3) *The Tracheal System*:—It seems clear that the original caudal processes could not have accommodated more longitudinal tracheæ than one each in the cerci (on the outer side), and a single pair in the appendix dorsalis. As this is the arrangement also in the filiform gills of the second larval instar (Text-fig. 34), we may take it as the primitive condition in the Zygoptera. When the Saccoid Type developed, each of these main tracheæ gave off a branch from the base; so that there were then two main tracheæ in each lateral gill, and four in the median. One of the two tracheæ in the lateral gills frequently divided a second time; so that the Saccoid Type, as it stands to-day, may show either two or three main tracheæ in the lateral gills.

From this primitive type, the Triquetro-quadrate Type developed, with four tracheæ still present in the median gill, and two in each lateral gill. Each of these numbers became reduced to one-half in the evolution of the Lestid form of gill.

From the Saccoid Type also, the Agrionid gill-forms were developed, with two tracheæ only in each of the three gills, both in the Vertical and Horizontal Types; *i.e.*, these lines of evolution only involved a reduction from four to two in the median gill, the number in the lateral gills remaining the same as before.

(4) *The Internal Laminae and Alveolar Meshwork*:—These must be regarded as developments peculiar to the Odonata. Probably both reached their maximum development in the spacious interior of the archaic Saccoid Type, with reductions or restrictions in later types, culminating in almost complete loss of both structures in the Lestid form of gill.

These changes may be readily exhibited in the following Table:—

TABLE OF PRINCIPAL DIFFERENCES IN INTERNAL STRUCTURE OF CAUDAL GILLS.

Group.	Blood-System.		Internal Laminae and Alveolar Meshwork.	Main Nerves.	Main Tracheae.
	Primary (Afferent) Canal.	Secondary (Efferent) Canal.			
Plecoptera ...	×	-	-	4, 2, 2,	0, 0, 0,
Ancestral Odonata ...	×	-	-	4, 2, 2,	2, 1, 1,
<i>Catolpe cygale</i> —					
Simple Saccus ...	×	×	×	4, 2, 2,	4, 3 or 2, 3 or 2,
Triquetri-quadrate ...	×	×	×	4, 2, 2,	4, 2, 2,
<i>Lesidae</i> —					
Vertical Lamella ...	×	×	almost absent.	2, 2, 2,	2, 1, 1,
<i>Agrionidae</i> —					
Constricted Saccus ...	×	×	×	4, 2, 2,	4, 2, 2,
Constricted Vertical Lamella ...	×	×	×	4, 2, 2,	2, 2, 2,
Nodate, etc., Vertical Lamellae ...	×	×	×	4, 2, 2,	2, 2, 2,
Horizontal Lamella ...	×	×	×	4, 2, 2,	2, 2, 2,
Reduced (Non-functional) Type ...	×	×	laminae absent, mesh-work present.	reduced or absent.	reduced.

In the above Table, × means present, - absent. In the last two columns, the first number stands for the condition in the median gill, the other two for that in the laterals.

Note on the Problem of the Origin of the Constricted or Two-jointed Gill:—The known facts for the solution of this incomplete problem are as follows:—

(1) In the Ontogeny of the Nodate and Subnodate Types (*Austroagrion* and *Ischnura*) no sign of a division of the gill into two parts is to be seen up to the seventh instar.

(2) In the fourth or fifth instar of *Neosticta*, the gill is a two-jointed or constricted saccus, but the distal joint is very small.

(3) Half-grown larvæ of *Caliagrion billinghami* (Nodate Type) show the constriction almost as marked as in *Isosticta*. These larvæ are probably of the tenth or eleventh instar.

Statements (1) and (3) might appear to be antagonistic; since, at the most, only four instars could intervene for the change from a simple to a constricted gill. But, as a matter of fact, the node in *Caliagrion* is much more strongly developed (in the full-grown larva) than it is in *Austroagrion*, though both are classed as of Nodate form. Consequently, if a series of instars of the rare larva of *Caliagrion* could be obtained, say, from the fourth upwards, the problem would very probably be solved. It would also be necessary to breed the larvæ of *Neosticta* or *Isosticta* from the egg, and raise them to the fourth or fifth instars, to complete the chain of evidence.

Thus we see that the solution of this interesting problem now rests only with him who can obtain two lots of very rare larvæ, and keep them alive through a sufficient number of instars. So far, I have not succeeded in doing this.